

Food Allergies

An Example of Translational Research



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KEYWORDS

• Food allergy • Translational • Mouse models • Allergy diagnosis • System biology

KEY POINTS

- Translational studies of food allergy include mouse studies, human studies, and computation methods and understanding how they complement each other.
- Two areas in which mouse studies complement human studies are in determining the immunologic processes sensitization and tolerance.
- Novel diagnosis approaches include component-resolved diagnostics, basophil activation test, histamine-release assays, mast cell activation test, and novel algorithms to predict the severity of reaction during oral food challenge.
- System biology approaches, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics, are being used in food allergy research.

INTRODUCTION

The Recent Rise of Food Allergies

Over the past few decades, IgE-mediated food allergy has become a chronic condition of increasing concern for clinicians, allergists/immunologists, and affected patients and their families. Food allergy constitutes a key step along the allergic march—the natural history of allergic disease manifestations that often progresses from allergic sensitization early in infancy to atopic dermatitis, food allergy, asthma, and allergic rhinitis. Although fatalities are rare relative to the number of people affected,¹ recent epidemiologic data suggest that the population-level burden of food allergy is substantial. A recent survey of a nationally representative sample of more than 50,000 US households estimated that approximately 8% of US children² and 10% of adults³ have a current food allergy—with 40% of affected children and 45% of adults allergic to multiple foods. When placed in the context of previous prevalence estimates, these data suggest that the prevalence of food allergies still may be

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on the rise in North America. A meta-regression of food allergy surveillance data collected by the US Centers for Disease Control and Prevention from 1988 to 2011 concluded that reported prevalence of pediatric food allergy had increased by 1.2 percentage points per decade (95% CI, 0.7–1.6).⁴ Given that a majority of patients with food allergy are not expected to naturally develop tolerance, particularly to the most common allergens (ie, peanut, tree nut, and seafood), it is likely that the prevalence of food allergies across the US general population is likely to continue rising until their etiology is better understood and effective prevention and/or treatment interventions are widely implemented.

The Recent Rise of Food Allergies—National Healthcare Utilization Data

The aforementioned cross-sectional survey data indicating rising food allergy prevalence in the United States are corroborated via examination of temporal trends in food allergy-related health care utilization. This includes a recent longitudinal analysis of national health insurance claims data,⁵ which demonstrated the incidence of peanut allergy increased steadily from 2001 to 2016. Another analysis of national claims data found that, from 2007 to 2016, the percent of claim lines with diagnoses of anaphylactic food reactions rose 377%.⁶ The most common eliciting allergic foods were peanut and tree nut, which are consistent with other US studies showing these as among the most frequent causative foods of severe allergic reactions and emergency department (ED) visits.⁷ Other studies of large private and publicly available⁸ administrative claims databases⁷ have found that rates of pediatric ED visits and hospitalizations for food-induced anaphylaxis have risen substantially over the past 2 decades. One study of the Healthcare Cost and Utilization Project Kids' Inpatient Database—the only all-payer pediatric inpatient care database in the United States—estimated a doubling of food-induced anaphylaxis hospitalization rates from 2000 to 2009.⁸ Another analysis of a private administrative care database concluded that rates of ED visits for food-induced anaphylaxis more than doubled from 2005 to 2014 among all segments of the US pediatric population—particularly among adolescents, where they rose more than 4-fold.⁷ Overall, the specific allergen responsible for the greatest increase in ED visitation was for reactions to tree nuts/seeds, rates of which increased more than 3.5-fold over the 9-year study period.

The Recent Rise of Food Allergies—Psychosocial Burden

In light of the relatively low fatality rates associated with food allergy and general lack of symptoms experienced by patients in the absence of acute allergenic food exposure—food allergies arguably impose their greatest burden in the psychosocial domain. Previous research describes how the stress of daily food allergy management compounded by a dearth of effective, accessible treatment options can have an adverse impact on family relationships and limit social activities, ultimately contributing to impaired food allergy-related quality of life.⁹ Previous studies have indicated that patients with a greater number of food allergies reported lower quality of life compared with their counterparts with fewer food allergies—likely a function of the greater degree of vigilance required for allergen avoidance.^{10–12} Among patients with specific allergies, those with milk and egg allergy report lower quality of life compared with children with more easily avoidable allergens, such as peanut and tree nut.^{13,14} Food allergy-related quality of life also is worse among children and caregivers for those with a history of severe food-allergic reactions, more symptoms during a previously reported food allergic reaction, and a prior history of epinephrine use.^{10,12,15} Similarly, individuals who believe themselves more likely to experience potentially life-threatening anaphylaxis report worse quality of life than their

counterparts who are less concerned about potentially fatal anaphylaxis. Due to the ubiquity of food, management of food allergies remains a daily challenge for many patients, as they seek to strike an appropriate balance between vigilance, preparedness, and stress management.

The Recent Rise of Food Allergies—Economic Impact

Beyond its effects on physical and psychological health, food allergy patients also incur substantial economic burden in their efforts to diagnose, treat, and manage this chronic condition on a daily basis. Although little is known about the economic burden of food allergies on adult patients, in 2013, the annual economic cost of pediatric food allergy was estimated to be \$24.8 billion, approximately \$4184 per child.¹⁶ Estimated annual direct medical costs totaled \$4.3 billion; annual out-of-pocket costs related to food allergy were \$5.5 billion; and annual opportunity costs, such as a caregiver leaving/changing jobs and missing work to care for their food-allergic child, were estimated to be \$14.2 billion. A 2019 systematic review of 11 studies addressing the economic burden of food allergy supported this conclusion that household-level lost opportunity costs appear to be a major driver of food allergy-related economic burden.¹⁷

Challenges Posed by the Rise of Food Allergies—Lack of Treatment Options

The rise of food allergies poses numerous challenges for patients and their families, chief among them is the current dearth of Food and Drug Administration (FDA)-approved treatment options. Although evidence for the safety and efficacy of numerous food allergen immunotherapeutic modalities continues to accumulate, as of Fall 2020, the only FDA-approved immunotherapy is an oral peanut allergy treatment—Palforzia—indicated for use in pediatric patients 4 years to 17 years of age. The epidemiologic data summarized previously, however, suggest that fewer than 1 in 10 US food allergy patients are peanut-allergic children (most are adults and/or allergic to allergens besides peanut). Furthermore, more than half of peanut allergy patients have multiple food allergies.

Challenges Posed by the Rise of Food Allergies—Lack of Understanding About Mechanisms

Although much has been learned about the etiology of food allergies in recent years,¹⁸ major knowledge gaps remain. For example, although recent data indicate that many adults develop new-onset allergies to foods in adulthood, which formerly were tolerated, it still is not fully understood how or why this occurs.³ Conversely, much remains unknown about the exact mechanisms through which some patients gain tolerance to foods that previously induced allergic reactions, whereas others remain allergic, and still others experience a progressive worsening of allergy symptoms. Improving scientific understanding of how to promote and sustain immune tolerance at molecular and cellular levels is fundamental to the success of emerging food allergen immunotherapies, including oral, sublingual, and epicutaneous approaches.

Challenges Posed by the Rise of Food Allergies—How to Prevent Them?

Over the past decade, findings from numerous studies investigating the safety and efficacy of earlier introduction of allergenic solids have been translated into revised guidelines for the prevention of peanut allergy.^{19–22} Much remains unknown, however, about the optimal timing, format, frequency, quantity, order, and/or dietary context (eg, probiotic, vitamin D, and/or fiber supplementation) in which “early” introduction of allergenic solids should occur to maximize preventive effects—particularly for allergens besides peanut. Although encouraging parents to incorporate allergenic solids

earlier and more often into their diet perhaps is the most widely studied food allergy prevention modality—others currently are being explored. These include (1) vitamin D supplementation among patients with vitamin D insufficiency; (2) aggressive, early emollient treatment of infant eczema (a key determinant of subsequent food allergy development)^{23,24} and other efforts to optimize early life skin barrier function; and (3) promoting exposure to a diverse array of environmental microbiota to help ensure colonization of the gastrointestinal tract by commensal microbes²⁵ believed to play a role in inducing a balanced, tolerogenic immune response.

Broad Areas of Study

Ongoing research into the molecular and cellular mechanisms of food allergy and immune tolerance is poised to provide key insights into why food allergies have apparently risen so dramatically in prevalence over recent decades. Improving understanding of how environmental influences interact with genetic risk to predispose individuals to developing food allergies has the potential to inform further advances in food allergy prevention and treatments. Moreover, learning more about predictors of both natural and treatment-induced tolerance to food allergens can help inform clinical management, including identification of patients who are most likely to potentially benefit from emerging immunotherapies.

Translating Food Allergy Research from Bench to Bedside

The National Center for Advancing Translational Sciences defines translation as “the process of turning observations in the laboratory, clinic and community into interventions that improve the health of individuals and the public—from diagnostics and therapeutics to medical procedures and behavioral changes.” The translational science spectrum ([Fig. 1](#))²⁶ shows the process of scientific research from knowledge about the biological basis of health and disease to delivery of interventions that improve the health of individuals and the public. The field of food allergy research currently is at an inflection point, where advances in cellular and molecular immunology are being translated into FDA-approved targeted food allergen immunotherapies and prevention interventions.²² This article aims to provide an overview of how cutting-edge research into the mechanisms of food allergy and immune tolerance currently is being translated and implemented in the clinical setting in order to reduce the public health burden of IgE-mediated food allergy.

OBSERVATIONS AND MECHANISMS

Mouse models are instrumental in deepening an understanding of immune mechanisms that human samples alone cannot accomplish. This section aims to demonstrate how mouse models can illuminate sensitization and tolerance mechanisms of food allergy.

Pros and Cons of Using Mouse Models

Awareness of the strengths and weaknesses of mouse studies facilitates a balanced interpretation of their results ([Table 1](#)).

Advantages of mouse modeling for food allergy studies:

1. Mouse models allow for tissue-specific analysis, such as lungs, gastrointestinal (GI) tract, and lymph nodes.
2. Mouse models provide mechanistic insight into food allergy sensitization, prevention, and persistence by identifying connections between essential cells, molecules, and conditions.

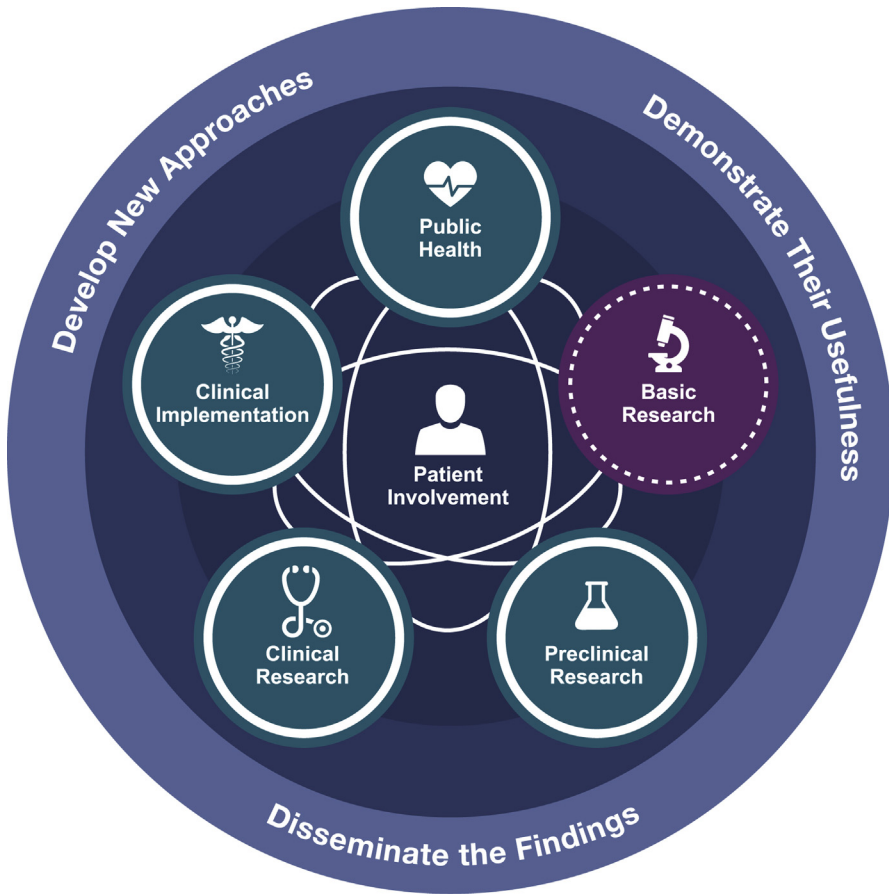


Fig. 1. The translational science spectrum illustrates the nonlinear, multidirectional process of scientific research from knowledge about the biological basis of health and disease to delivery of interventions that improve the health of individuals and the public. (From the NIH National Center for Advancing Translational Sciences (NCATS); with permission.)

3. Genetic models allow for specific studies on the roles of specific cell types and cytokines by inducing mutations, such as gain of function, loss of function, and conditional. Transfer of specific cells or microbes also is useful.
4. Mice live in carefully controlled conditions, reducing confounding factors inherent in human studies.

Some disadvantages include

1. Mouse models may not represent human disease accurately.
2. Several colliding factors likely are responsible for food allergy, such as environment, genetics, diet, and microbiome changes.^{27,28} Oversimplification and overestimation of individual factors are likely to minimize other factors' roles.
 - a. Allergic disease processes may be different in mice than in humans due to biological differences.

Table 1 Strengths and weaknesses of using mouse models for food allergy	
Strength	Weakness
Allows for tissue-specific analysis, such as lungs, gastrointestinal tract, and lymph nodes.	The model may not accurately represent human disease.
Provides mechanistic insight into food allergy sensitization, prevention, and persistence by identifying connections between essential cells, molecules, and conditions	Several colliding factors are responsible for food allergy, such as environment, genetic, diet, and microbiome changes. ^{27,28} Oversimplification and overestimation of individual factors are likely to minimize other factors' roles.
Genetic models allow for specific studies on the roles of specific cell types and cytokines by inducing mutations, such as gain of function, loss of function, and conditional. Transfer of specific cells or microbes also are useful.	
Mice live in carefully controlled conditions, reducing confounding factors inherent in human studies.	

- b. Human behavior at the individual, family, and societal levels is critical to understanding food allergy etiology such that mouse studies cannot provide the insights necessary to address the food allergy epidemic fully.

Translation—modeling human disease

Key areas that mouse studies contribute to understanding mechanisms of tolerance and sensitization to food allergens are studies investigating the microbiome and route of allergen exposure.

Microbiome

The microbiome is a vital piece of the food allergy puzzle. A healthy microbiome helps prevent food allergy.^{18,27,28} *Clostridia* species have several benefits to human gut health²⁹ and mouse studies flesh out potential mechanisms. For example, a mouse study showed that *Clostridia* species promote gut integrity by inducing interleukin (IL)-22 secretion from innate lymphoid cells and helper T cells (T_H), thereby regulating intestinal epithelial permeability.³⁰ *Clostridia*-enriched microbiota transferred into germ-free mice induce higher levels of Foxp3 expressing regulatory T cells (Tregs) in the colon, which helps promote tolerance.¹⁸ Certain *Clostridia* species promote a transforming growth factor (TGF)- β and IL-10-rich cytokine milieu in mouse colons, essential for a tolerogenic gut environment.³¹

Clostridia also have therapeutic benefits for at least 2 food allergy models. For example, introduction of *Clostridia* species into gnotobiotic mice reduced levels of peanut-specific and total IgE relative to germ-free controls.²⁹ Another study examined whether commensal bacteria play a causal role in the cow's milk allergy (CMA) allergic response. Investigators colonized germ-free mice with feces from infants with CMA or healthy infants and sensitized them to β -lactoglobulin. Sensitization occurred in the CMA colonized mice but not the healthy control mice. The investigators identified the *Clostridia* species *Anaerostipes caccae* as a protective species that may be therapeutically relevant for infants with CMA.³²

Certain bacteria also play a role in sensitization to food allergy. Mice with gain of function mutations in the IL-4 α subunit have elevated STAT-6 signaling of IL-4 and IL-13, leading to increased food allergy susceptibility. Food allergy sensitized mice show altered abundance of the bacterial species *Lactobacillaceae*, *Rikenellaceae*, and *Porphyromonadaceae*.³³ Transfer of the microbiota from these mice into germ-free mice transferred increased susceptibility of food-mediated anaphylaxis.

The surface has barely been scratched on the microbiome's contribution to food allergy. Further research using mouse models will allow researchers to identify other beneficial and harmful microbes, their mechanisms, and how they translate to human disease.

Environmental exposure in sensitization

Current hypotheses suggest that the route of initial bodily exposure to food allergens is a significant contributor to sensitization or tolerance. Initial allergen exposure via nonoral routes, such as through the airway or skin, is likely to lead to sensitization. In contrast, initial exposure via oral consumption is likely to be tolerogenic.^{19,34} Models are needed that take these observations into account.

Mouse models are most effective when they model the human condition as closely as possible. An excellent example of this are models investigating peanut allergy.

Multiple mouse studies have examined peanut skin sensitization by epicutaneous exposure on the ear pinna of mice with crude peanut extract or the peanut protein components Ara h1 and Ara h2 or by utilizing abdominal tape stripping and peanut flour exposure to the tape stripped area.^{35,36} Sensitization also occurs through airway peanut exposure.³⁷ In each study, sensitized mice exhibit elevated peanut-IgE levels and induction of anaphylaxis upon peanut protein challenge, thus modeling the human condition. None of these models uses adjuvants and likely is closer to how environmental exposure occurs in infants.

Type 2 helper T cells and follicular helper T cells drive peanut-specific antibody responses and anaphylaxis

Both the airway and tape-stripping with peanut flour models demonstrate that sensitization to peanut depends on follicular T_H (T_{FH}), whereas the ear pinna sensitization model indicates T_H2 cells are required for sensitization. As the tape stripping skin and airway models show, T_{FH} cells are essential for peanut-IgE and anaphylaxis. This likely is because T_{FH} cells are specialized to interact with germinal center B cells in the lymph nodes, thus generating somatic hypermutation and affinity maturation.³⁸ Additionally, IL-13 derived from type 2 innate lymphoid cells is indispensable for T_{FH} cells generation and plasma peanut-IgE.³⁹

The method and route of sensitization and allergen itself may induce differing immune pathways because the use of specific proteins leaves out other components of peanut, such as fats and carbohydrates. Although the peanut proteins Ara h1, Ara h2, Ara h3, and Ara h6 are the main targets of peanut-specific antibody responses, peanut is a complex molecule, and the other components may have unique roles in initiating the direction of the immune response.

Tolerance

Models of mouse tolerance can help define mechanisms of tolerance generated and loss. Foods typically are well tolerated; however, a breakdown of tolerance to the same food sensitizes, thus acting as either an agent of sensitization or tolerance. The phenomenon of oral tolerance has long been an area of scientific interest. In 1907, guinea pigs' sensitization to vegetable proteins resulted in violent anaphylaxis

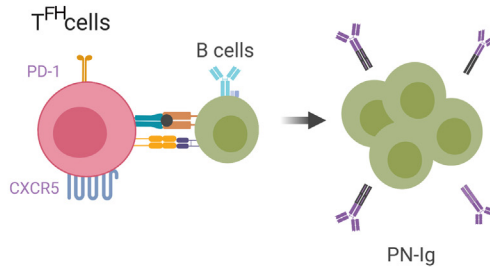
and death.⁴⁰ In 1911, Wells⁴¹ demonstrated that guinea pigs raised on a daily diet of corn before sensitization protected them from anaphylaxis and death. Since then, several studies have assessed how tolerance develops. Many of these studies used the protein ovalbumin (OVA) with an adjuvant.^{42–45} Whether these observations from OVA hold for real-world food allergens is unknown. OVA is not a natural allergen; sensitization can only occur when OVA is coupled to an adjuvant, or by use of transgenic mice with genetically modified T-cell receptors (TCRs) specifically engineered to react against OVA peptides. Real-world allergens are more biologically complex, and tolerance induction may differ depending on the allergen's nature.

A mouse model can examine how oral tolerance protects from sensitization, both immunologic and clinically. For example, using a model of milk casein allergy, Kim and colleagues⁴⁶ showed that IL-10-producing B-regulatory cells from the mesenteric lymph nodes, but not from the spleen of peritoneal lymph nodes, protected mice from casein-induced allergic responses through Tregs. Another study showed that feeding mice peanut butter before skin or airway exposure mice protected them from sensitization, mirroring the Learning Early About Peanut (LEAP) study results.⁴⁷ Examining T cells in the lung draining lymph nodes revealed that tolerance was dependent on the inhibitory molecule CTLA-4 (Fig. 2).

Conclusions and future directions of mouse modeling

Mouse studies have helped generate novel ideas and insights that translate well to human food allergy. Areas in which mouse studies have helped significantly are the microbiome's role and how environmental exposure has an impact on immunologic mechanisms of sensitization and tolerance.

Skin or airway T-cell-mediated sensitization to peanut



Oral-induced T-cell-mediated tolerance to peanut

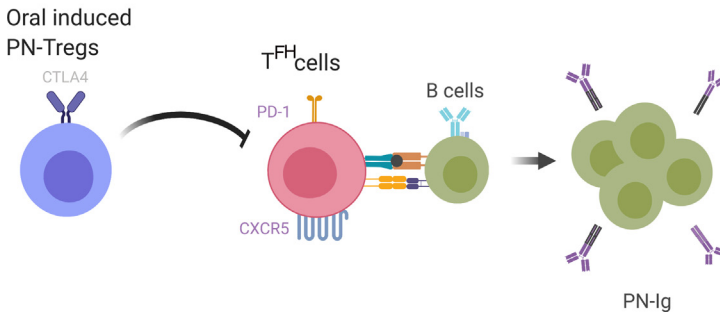


Fig. 2. T-cell-mediated sensitization and tolerance.

Clostridia species appear to promote immune tolerance, whereas sensitized mice demonstrate alterations in *Lactobacillaceae*, *Rikenellaceae*, and *Porphyromonadaceae* species. Further work to clarify beneficial and harmful species is needed.

Mouse work also helps confirm the hypothesis that allergen exposure's initial route plays a significant role in sensitization and tolerance. Mice exposed to peanut via the nonoral skin or airway routes are sensitized. At the same time, tolerance occurs when mice are exposed to peanut butter orally before skin or airway exposure. Mouse modeling also demonstrated immunologic mechanisms of sensitization and tolerance. Key roles for T_{FH} cells, CTLA4-expressing Tregs, and IL-10-producing B-regulatory cells are described.

Mouse models can continue to assist in other areas of food allergy. The initiating events of how allergens engage with innate immunity are unknown. Appropriate mouse models can help clarify these early events in a way that human samples cannot. Insights from these studies may help provide ideas for therapeutics to stave off food allergy in at-risk children.

It is unknown how food allergies persist. For example, the contributions of memory T cells, memory B cells, plasma cells, and microbes are unknown in maintaining food allergy. These studies are well suited for mouse modeling because many studies may involve tissue-specific analysis and bone marrow extraction. Insight from these studies likely would generate novel directions in therapeutic ideas to reverse sensitization.

Lastly, the effectiveness of mouse modeling in food allergy is related directly to how well it models the human condition.

Mechanisms learned from human studies

IgE-mediated food allergy accounts for the vast majority of food-induced allergic reactions.

The knowledge of mechanisms behind the induced desensitization in allergen immunotherapy has been advancing largely through investigating peripheral blood of human patients undergoing oral immunotherapy (OIT) trials, with changes occurred at local tissues including gastrointestinal tract and skin barrier as peripheral subjects of interest.

This section focuses on findings emerged in recent human studies of food allergy, including OIT trials.

Timing of allergen introduction

For decades, allergen avoidance remained the standard clinical practice of preventing food allergies. Since the American Academy of Pediatrics issued a revised infant feeding policy in 2008,⁴⁸ early introduction of highly allergenic foods has been increasingly recommended for all infants. Results from the LEAP trial demonstrated early introduction of peanut allergen in high-risk infants can reduce the risk of developing peanut allergy.¹⁹ A meta-analysis of 5 trials showed that early egg introduction at 4 months to 6 months was associated with reduced egg allergy.⁴⁹ New clinical guidelines are under implementation, with physicians facing challenges in offering evidence-based recommendations.⁵⁰

Immunoglobulins and basophils

The key factor that inhibits effector cell activation mediated by IgE comes from an increase in allergen-specific IgG4 level, which is a common feature of and a response to allergen immunotherapy.⁵¹ Although allergen-specific IgE (sIgE) level is not a predictive biomarker to clinical reactivity,⁵¹ IgE antibodies to Ara h2 have been reported to efficiently differentiate clinical peanut allergy from asymptomatic peanut

sensitization.⁵² Lower peanut-induced basophil activation and higher peanut allergen-specific IgG4/sIgE before treatment is associated with OIT-induced sustained effects in patients undergoing a double-blind, randomized, placebo-controlled, phase 2 peanut OIT trial.^{53,54}

T cells

Activation and polarization of T-cell subsets are the main effectors in allergic inflammation and tolerance. Desensitization to allergens often is associated with a shift away from a T_H2-skewed phenotype toward a T_H1-skewed response, together with restored suppressor function of allergen-specific Tregs, as reported in multiple reports of OIT clinical trials.^{55,56} Expression of skin and gut homing receptors on the surface of allergen-specific T cells has been found to increase in patients with milk allergy and other atopic conditions.^{57,58}

Microbiome and epithelial barrier

There has been an increase of interest in studying the interplay between commensal bacteria and the gastrointestinal tract mucosal surface, which serves as the first physical layer of protection against foreign antigens. Intestinal bacteria are now known to play a role in regulation allergic responses to dietary antigens. Intestinal bacteria of healthy infants have demonstrated a protective effect against anaphylactic responses to a cow's milk allergen in colonized mice.⁵⁹ As a recent report showed in peanut-allergic adult patients, an increase of gut microbiota diversity was observed during active oral immunotherapy.⁶⁰

DIAGNOSIS: CHALLENGES AND FUTURE PROSPECTS

A safe and accurate diagnosis of food allergy is extremely important to guide safe and yet not overly restrictive dietary management. Currently, the cornerstone of the diagnosis of IgE-mediated allergy is the clinical history followed by in vitro or in vivo tests, including the skin prick test (SPT), sIgE test, cellular test, and oral food challenges (OFCs).⁶¹ These methods of diagnostic testing for allergy often are inconclusive and, in cases of food challenges, sometimes stressful and scary. **Table 2** shows the advantages and disadvantages of diagnostic approaches. Researchers are trying to identify more safe and precise diagnostic methods, including developing new approaches, improving current tests, and combining diagnostic tools together to improve the accuracy of the predictive performance of diagnosis.

Double-blind placebo-controlled food challenge (DBPCFC) still is the only gold standard for definitive diagnosis and sometimes cannot be avoided,⁶² except when a patient has suffered an anaphylactic shock. OFC, however, is time-consuming, costly, and burdened by the risk of life-threatening anaphylactic reactions.^{62,63} Therefore, reliable prognostic markers for predicting severity of allergic reactions during OFC are needed.^{64,65} Chinthrajah and colleagues⁶⁶ proposed an integrated approach combining laboratory values (ratio of peanut-stimulated basophils to anti-IgE-stimulated basophils), along with clinical variables (exercise-induced asthma and forced expiratory volume in the first second of expiration/forced vital capacity ratio at time of DBPCFC) to be incorporated into a novel algorithm for assigning challenge severity score to predict the severity of reaction during peanut OFC. This decision rule is under a clinical trial (NCT02103270) for further testing before it can be considered outside research settings.

For many patients, conventional allergy tests like SPT and sIgE to allergen extracts that indicate they might react to a food are misinterpreted to mean that they do react to that food. This can lead to unnecessary avoidance of foods, which increases costs,

Table 2

Advantages and disadvantages of diagnostic approaches for food allergy

Diagnosis Approaches			Advantages		Disadvantages
Conventional approaches	SPT	Total IgE, specific-IgE	Fast; cheap; no risk of allergen exposure		High false-positive rate
	Serum IgE test OFC		Fast; no risk of allergen exposure The only gold standard for definitive diagnosis		High false-positive rate Time-consuming; costly; risk of life-threatening anaphylactic reactions
Novel approaches	CRD	Single or multiplex molecular allergens assays	More accurate diagnosis, capable of discriminating cosensitization vs cross-sensitization phenomena		CRD offered increased specificity but decreased sensitivity, when compared with traditional SFT and serum-specific IgE testing
	Cellular test	BAT	Shows advantage compared with HRA and MAT		BAT requires fresh blood samples for testing; not applicable for subjects with low basophil number
		MAT	Adds significant diagnostic value to conventional methods and reduces the need for OFC testing; no risk of allergen exposure	Using serum instead of fresh blood; applicable to subjects whose basophils do not respond to the BAT	Lower sensitivity than BAT
		HRAs		Diagnoses subjects with low basophil number in contrast to HRA	Lower sensitivity than BAT
	Novel algorithm	Novel algorithm to predict the severity of reaction during peanut OFC	Reduces the risk of life-threatening anaphylactic reactions caused by OFC		Might need further testing and optimization

adds to anxiety, and limits food choices and nutrition options. To improve current diagnostic tests, researchers are trying to use pure allergens instead of food allergen extracts for the SPT and sIgE tests. With pure allergens, it is possible to overcome the problem of low concentration and low stability of the allergens that may be lost when making a food allergen extract. The advanced antigen-based tests like component-resolved diagnostics (CRD) characterize the molecular components of each allergen involved in a sIgE-mediated response. In the clinical practice, CRD can lead to more accurate diagnosis and selection of therapeutic intervention^{67,68} and increasingly is incorporated into clinical workflows.⁶⁹ A recent systemic review suggested that sIgE to Ara h2 can enhance the certainty of diagnosis and reduce the number of OFCs necessary to rule out clinical peanut allergy in unclear cases.⁷⁰ A Europe-wide hazelnut allergy study suggested that combinations of CRD-based approaches with clinical history and extract data are superior to CRD alone.⁷¹ Another advanced technology for allergy diagnosis based on CRD is multiplex molecular allergens assays, such as Madx ALEX⁷² and Euroimmun Multiplex immunoblot assay,⁷³ which enable clinicians to simultaneously detect multiple sIgE test in a single test that requires only a small amount of blood. This will be useful particularly for younger children from whom only small amounts of blood may be taken for testing. The multiplex CRD also will enable clinicians to discriminate cosensitization versus cross-sensitization phenomena and can be useful to stratify the clinical risk associated with a specific sensitization pattern, in addition to the OFC.

Another recent area of research is to improve the allergy diagnosis by using cellular allergy tests, such as the basophil activation test (BAT),^{74,75} histamine-release assays (HRAs),⁷⁶ and, more recently, the mast cell activation test (MAT),^{77,78} which use cell activity as a measure of allergic responsiveness. Those tests have been reported to add significant diagnostic value to SPT and IgE-based test methods and reduce the need for OFC testing. BAT was compared with histamine release (HR) and passive HR and did not show a significant advantage but could diagnose subjects with low basophil number in contrast to HR.⁷⁶ The reports on the usage of passive sensitization strategies using the mast cell lines¹² or mast cell precursors¹³ showed promising potential on distinguishing peanut allergy from peanut sensitization but lower sensitivity than BAT. MAT has the advantage, however, of using serum instead of fresh blood, overcoming the limitation that BATs require fresh blood. In addition, the MAT provides results for patients whose basophils do not respond to the BAT.

Although an increasing number of advanced diagnostic methods are under development, no currently available tests can predict the severity of a patient's next food allergy reaction. Identifying new diagnostic tests that are easy, safe, accurate, and stress-free still is an unmet need. Novel diagnostic methods integrating data from different sources and technologies together with a better understanding of mechanisms of allergic inflammation may contribute to developing sophisticated precision medicine tools for better diagnosis.

FOOD ALLERGEN AND SYSTEM BIOLOGY

Another example of translational research in food allergy is the application of omics technologies and systems biology approaches. Systems biology studies biological process through comprehensive interrogations of the biomolecules at different levels to gain insights into complex biological systems⁷⁹ (Fig. 3). In food allergy, multiple players at different scales, including food proteins, gut microbiome, host gastrointestinal tract, host immune system, and more, interact with each other dynamically and contribute to the process. The high individual heterogeneity and complex nature of

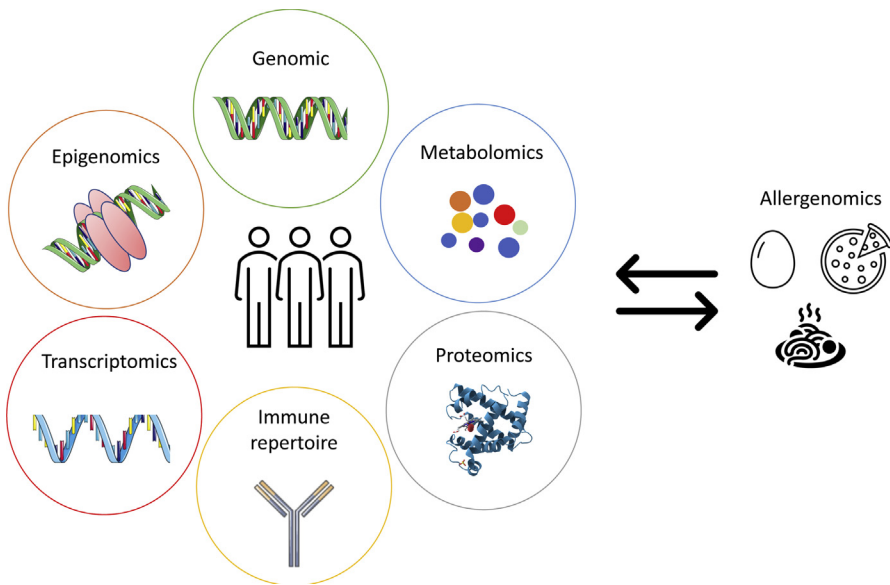


Fig. 3. Genomic, epigenomic, transcriptomics, metabolomics, proteomics, and immune studies are part of a comprehensive interrogations of the biomolecules at different levels to gain insight of the complex biological system to study food allergies.

food allergy make it an especially suitable target for systemic biological approaches. With the rapid advance of omics technologies, massive data now can be generated at relatively low cost. Systemic approaches have been used to not only better understand the mechanisms behind food allergy but also show potential for identifying biomarkers to better diagnose, prevent, and treat food allergy.⁸⁰ Omics technologies, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics, have been or are being used in food allergy research (Table 3).⁸¹

Although the contribution of heritable factors to food allergy remains to be determined, a body of evidence has identified certain genetic predispositions to food allergy. In genomic-wide association studies, genetic variants, typically single-nucleotide polymorphisms (SNPs), are examined across the whole genome. Several loci have been linked to the risk of food allergy.⁸² SNPs mapping to the HLA regions have been shown to be associated with peanut allergy⁸³ and wheat allergy.⁸⁴ Genetic background also was found to be associated with total IgE level as well as sIgE levels.⁸³ Immune repertoire, referring to the genetic diversity of B-cell receptors (ie, immunoglobulins) and TCRs, also is under intense investigation in food allergy. Studies of IgE-producing B-cell receptors have revealed the mechanism of IgE class switching during allergen-specific immune responses.^{85,86} Another study found certain TCR clones in CD4⁺ T cells that recognized peanut epitopes were shared among peanut allergic individuals.⁸⁷

Epigenetic modifications, including DNA methylation, histone modification, and nucleosome positioning, induced by environmental factors, can affect the expression profiles of the genomes.⁸⁸ The increasing risk of food allergy in the recent generations could be linked with environmental changes through epigenetic modifications. One study of Dutch children found that DNA methylation generally was increased with CMA.⁸⁹ Peanut allergic individuals who were desensitized through immunotherapy had hypomethylation of FOXP3 gene in Tregs compared with those who were nontolerant.⁹⁰

Table 3
Genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics applied to food allergy

Platforms	Targets	Application Examples in Food Allergy
Genomics	SNPs across genome	SNPs at HLA regions associated with food allergy
Immune Repertoire	TCR and B-cell receptor diversities	Identified origin of IgE-producing B cells; identified common TCR recognizing peanut
Epigenomics	DNA methylation, histone modification, and nucleosome positioning	DNA methylation associated with CMA
Transcriptomics	Gene expression profiles	Immune gene clusters were associated with allergic response in peanut allergy.
Proteomics	Protein expression at cellular level	Identify immune cells population and functions associated with allergy response
Metabolomics	Metabolites and metabolic pathway in host and microbes	Food allergy associated with decreased sphingolipid levels in serum
Allergenomics	Protein/peptides in the food	Identify allergens and epitopes

Transcriptomics examine the RNA molecules and provide the information of the current gene expression profile of the sample. Immune cells, usually from peripheral blood samples, from allergic individuals were isolated with or without the stimulation of relevant food allergens and analyzed for gene expressions. Gene clusters related to acute and inflammatory pathway were identified in peanut allergic children following OFCs.⁹¹ Single-cell RNA sequencing also was applied to examine circulating immune cells before and during OIT for peanut allergy and observed a transient increase of TGF- β -producing cells and changes in T_H2 cells.⁹²

Proteomics aim to measure all the protein components in the samples. Mass cytometry detects heavy metal ions instead of fluorescent dyes in the traditional flow cytometry and allows for measuring more than 40 protein markers simultaneously within a single cell.⁹³ This permits more detailed and efficient investigation of immune cells than allowed by previous approaches. In peanut allergy, investigators have found increased frequency of activated B cells and peanut-specific memory CD4 T cells using mass cytometry.⁹⁴ Another application of proteomics in food allergy is to identify and characterize allergens in the offending food using mass spectrometry, also called allergenomics, which is important in food safety.⁹⁵

Metabolomics measure the collection of metabolites (generally small molecules) including sugar, lipids, amino acids, fatty acids, and so forth. This is an emerging field and studies applying metabolomic approaches to food allergy are relatively scarce. In an asthma/food allergy pediatric cohort, Crestani and colleagues⁹⁶ found food allergy was uniquely associated with decreased sphingolipid levels in serum. Metabolomics also is particularly interesting to consider in combination with microbiomics approaches because microbiota may affect host immune system through metabolites they secrete. (The microbiome in food allergy are discussed extensively previously.)

Despite the decreasing cost of omics technologies, the implications of omics technologies still are mainly in the research setting because these technologies generally

require highly trained expertise to both acquire and analyze data.⁸⁰ Automated sample processing and artificial intelligence-aided data analysis may make these tools more available in the future within routine clinical settings. The application of systemic biology in food allergy can lead to identifications of novel biomarkers and hints to inspire new prevention strategy or treatments.

CLINICS CARE POINTS

- Food allergies are among allergic disorders, including atopic dermatitis, asthma, and allergic rhinitis. Many individuals with a single allergic disorder eventually develop other atopic comorbidities, suggesting common underlying mechanisms. Classically, the earliest manifestation of allergic diseases starts with atopic dermatitis in infancy and preschool years, and these individuals then may develop food allergy, allergic rhinitis, and/or asthma in subsequent years.^{97,98} This natural progression of allergic disorders is termed the allergic or atopic march.
- Food allergies are adverse antigen-specific immune-mediated responses upon exposure to a given food, affecting 8% of children and 5% of adults in the United States.⁹⁹ Common allergenic foods include milk, eggs, peanuts, tree nuts (such as almonds, cashews, and walnuts), fish, shellfish, soy, and wheat. Prevalence of peanut allergy is estimated to be 2% to 3%.^{100–102} IgE-mediated food allergies are characterized by the rapid onset of symptoms (typically observed within minutes to approximately 2 hours) after ingestion of the suspected allergen, generally proteins in foods.¹⁰³ Food allergy reactions can lead to anaphylaxis, a rapid and systemic allergic reaction, involving the upper and lower airways, skin, conjunctiva, gastrointestinal tract, and cardiovascular systems. Untreated, anaphylaxis can be fatal, most often due to upper or lower airways obstruction or to cardiovascular collapse.^{103,104} Cofactors, such as concomitant infection, medication, alcohol intake, and exercise, may influence the occurrence of anaphylaxis.
- Other subtypes of food allergy include oral allergy syndrome, a hypersensitivity reaction to plant-based foods, usually mild, due to cross-reactivity between food proteins and inhalant allergens, with usually mild symptoms (eg, PR-10 syndrome); alpha-gal allergy, a reaction to an oligosaccharide present in red meats and tick saliva¹⁰⁵; eosinophilic esophagitis, a chronic, local, immune-mediated esophageal disease,¹⁰³ whose pathophysiology is unclear but involves food allergy¹⁰⁶; and non-IgE-mediated food allergies, such as food protein-induced allergic proctitis, food protein-induced enteropathy, food protein-induced enterocolitis.
- Until recently, the only effective therapy for food allergy was strict avoidance of the allergenic food. New approaches with disease-modifying properties, including allergen immunotherapy and the use of biologics, most of which are humanized monoclonal antibodies targeting the T_H2 inflammatory pathway, have been developed in the past 20 years, leading to a revolution in the field.¹⁰⁷ Preventive strategies to limit risk factors and their impact on the mechanism of food allergy also are encouraged by a growing body of research. The most effective preventive strategy might be early introduction of solid foods at 4 months to 6 months of age, and guidelines no longer recommend allergen avoidance.¹⁰⁸ Consistent with the dual-allergen hypothesis and atopic march, prevention of allergen exposure through maintaining skin barrier integrity also could serve as an effective preventive strategy in the future. Finally, findings on the role of the microbiota in allergic disorders and immune tolerance have led to the idea of probiotic supplementation as a preventive intervention, although current evidence does not indicate that probiotic supplementation reduces the risk of developing food allergy in children, as opposed to a possible benefit in the primary prevention of atopic dermatitis.¹⁰⁹
- From bench to bedside, fully understanding the mechanisms leading to food allergy and tolerance will provide deeper comprehension of the relevant clinical outcomes in food allergy patients. Studying the modifications induced by novel therapeutics may improve overall comprehension of their mechanisms and allow introducing them in the standard of care.

DISCLOSURE

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